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CONTRIBUTION TO THE LIFE HISTORY OF SALIX.[1]

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(WITH PLATES XII-XVIII)

MANY considerations combine to make the embryology of Salix an inviting subject. Taxonomists at present place it so near the most primitive dicotyls that it becomes interesting from a phylogenetic standpoint. Treub's researches upon Casuarina (22) have yielded some remarkable results. He finds that it has a great number of macrospores, but no synergids and no antipodals; that there is no primary endosperm nucleus formed by the fusion of polar nuclei, but an endosperm formed before fertilization; that cell walls are formed about the oosphere and its accompanying cells before fertilization; and finally that the pollen tube enters by way of the chalaza instead of the micropyle. Treub considered these results so significant that he proposed a primary division of angiosperms into chalazogams and porogams, Casuarina being the sole representative of the former. The researches of Nawaschin (25) and Miss Benson (26) have disproved the taxonomic value of chalazogamy, but the unique structures of the Casuarina embryo sac have not been discoverea as yet in any other plant.

With such discoveries among the lower dicotyls, Salix might

[1] Contributions from the Hull Botanical Laboratory. III.—The previous Contributions are, I. "The embryo sac of Aster Novæ-Angliæ," by Chas. J. Chamberlain, BOT. GAZETTE **20**: 205–212. *pl. 15, 16.* 1895; II. "Notes on the fertilization and embryogeny of Conifers," by John M. Coulter, BOT. GAZETTE **23**: 40–43. *pl. 6.* 1897.

be expected to prove instructive. The perplexing variation in species, the well known propensity to hybridize, and the frequency of sports increase the probability of interesting results. Finally, Chicago and its environs afford an abundance of material representing three-fourths of the species credited to the United States.

At first it was my purpose to examine Salix only with reference to chalazogamy and the structures of the embryo sac, but as the subject developed it was thought best to extend the scope of the work. The subjects discussed are (1) material and methods, (2) organogeny of the flower, (3) development of the microspores, (4) origin of the macrospore, (5) germination of the macrospore, (6) pollen tubes and fertilization, (7) development of the embryo, (8) teratology, (9) Salix and other Amentiferæ, (10) summary.

Complete series from the formation of the archesporium to the mature embryo were studied in *S. petiolaris* and *S. glaucophylla*. Series lacking but few stages were studied in *S. tristis*, *S. discolor* and *S. cordata*. Less complete series were studied in twelve other species.

The investigations were conducted under the guidance of Professor John M. Coulter, whose valuable suggestions and kindly encouragement are acknowledged with gratitude.

MATERIAL AND METHODS.

The greater part of my material was collected at Grand Crossing, Illinois, but many gatherings were made from the higher ground north of Chicago, and from the sand dune region of northern Indiana. The collecting began February 14, 1895, and gatherings were made at intervals of two or three days until the latter part of May. A few collections of buds were made in the autumn and winter. This furnished nearly complete series in *S. glaucophylla*, *S. cordata*, and *S. tristis*, with many other species represented by several stages. During the following spring gaps in the series were filled, a good series of *S. petiolaris* was collected, and many monstrous forms were found. In August and

December collections were made to determine the histological character of the winter buds.

At first 1 per cent. chromic acid was used for killing and fixing, but experience proved that better results could be obtained by adding a little acetic acid to counteract the tendency to shrink. The material was left in the fixing agent 12 to 24 hours, then washed in water for 24 to 36 hours, and after passing through successive grades of alcohol was left in 70 per cent. alcohol until needed for use. Flemming's fluid proved excellent, and the same must be said of Merkel's and Hermann's, but these are rather expensive. Picric acid with a trace of acetic is also to be recommended. To insure rapid fixing the tops of many of the pistils were cut off down to the level of the ovules.

Xylol proved the best clearing agent. The transfer from absolute alcohol to xylol was made gradually by adding small quantities of xylol to the alcohol until the mixture contained about three parts of xylol. The mixture was then poured off, and pure xylol was substituted. As soon as the material was cleared, a lump of paraffin was added, and thus the transfer from xylol to paraffin was made gradual. One to three hours in the bath is sufficient after such treatment.

Serial sections were cut with a Thoma microtome. Mayer's albumen fixative in connection with the water method was used for fixing the sections to the slide. Cyanin and erythrosin was the best combination for embryo sacs. Delafield's hæmatoxylin was good for embryos and the early stages of anthers. Safranin and gentian violet, cleared in clove oil, seems to be the best combination for the pollen grain.

The celloidin method was tested, but did not give as good results as paraffin, and besides was unnecessarily tedious.

All drawings were made with an Abbé camera lucida. A $\frac{1}{12}$ Bausch and Lomb immersion was used for all the drawings except those of *pl. XVIII* and *fig. 66a.*

ORGANOGENY OF THE FLOWER.

No attempt was made to secure a perfect series of stages in the development of the floral organs. Several species were col-

lected in August to determine the condition of the buds. In some of these the carpels appeared as slight protuberances; others were more advanced and showed the carpels outlined but with no trace of ovules. In October buds of *S. cordata* and *S. glaucophylla* the nucellus of the ovule was quite conspicuous, but the integument had not begun to form. As a rule, the integument does not form until spring. Early February buds from a small plant of *S. cordata* showed carpels but no trace of ovules. Material from the same plant taken three weeks later showed a conspicuous integument. Staminate buds, collected in October, showed the stamens fairly outlined. The gland, or nectary, is frequently conspicuous in the winter buds.

A diligent search was made for rudiments of floral organs which might be expected to be found were the flowers of Salix reduced rather than primitive, but an examination of early stages in several species failed to show the least trace of anything which could be interpreted as a petal or sepal, or as indicating an earlier ambisporangiate condition. The prominent nectar gland has a single terminal pore. There is nothing in its history which would allow it to be regarded as a reduced or transformed floral organ.

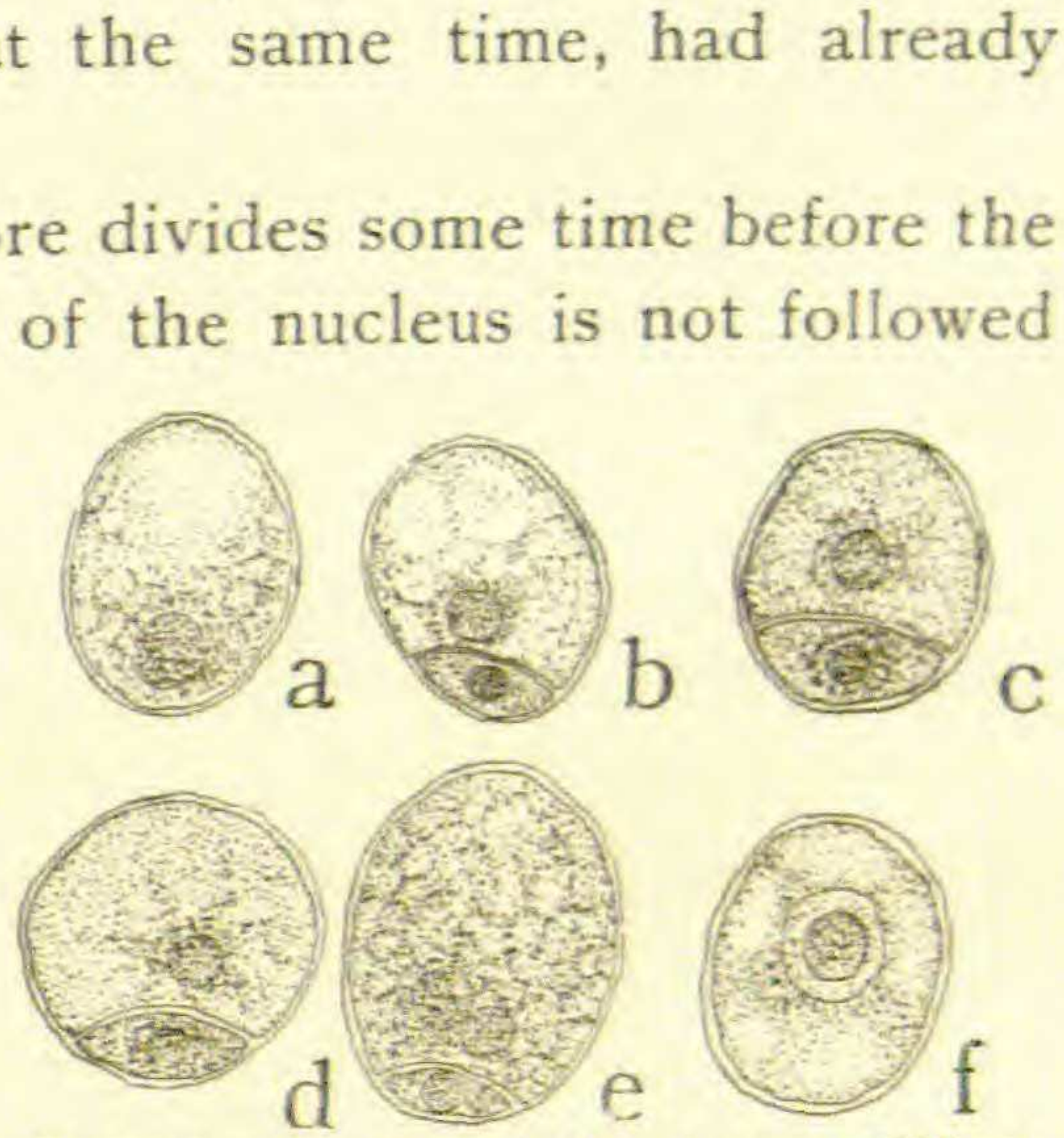
DEVELOPMENT OF THE MICROSPORES.

Staminate buds of *S. glaucophylla*, collected early in October, showed the condition represented in *fig. 1*. There are here three layers of cells between the epidermis and the sporogenous cells. The three layers appear alike, the endothecium and tapetum having no distinguishing characters. In some cases there were four layers instead of three. Another specimen of *S. glaucophylla*, collected at the same time, had the tapetum somewhat differentiated. That buds pass the winter in about this state is proved by the fact that buds of the same species collected in midwinter showed the same condition. Material of *S. tristis*, collected late in March, showed the tapetum well differentiated, but the endothecium still appearing like the middle layers (*fig. 2*). The number of middle layers may vary from one to four, even

in the same anther. In *S. glaucophylla* and *S. tristis* the number of these intervening layers is usually two. Some anthers of *S. cordata* with the pollen grains nearly mature showed no layer at all between the endothecium and the tapetum (*fig. 8*). The cells of the mature tapetum often have two nuclei. The strengthening of the endothecium does not commence until the tapetum has begun to disorganize. All of the layers between the endothecium and the spores disintegrate, and the spores float in a granular fluid (*fig. 9*).

The sporogenous cells, as shown in *figs. 1* and *2*, are the mother cells of the microspores. This is proved by the fact that the number of sporogenous cells in a transverse section of an autumn or winter microsporangium is approximately the same as the number found in spring microsporangia whose sporogenous cells are beginning to show by their spherical form that they are undoubted mother cells. The large size of the nuclei also favors this interpretation. An examination of several species indicates that most staminate buds pass the winter in the spore mother cell stage. In buds of *S. tristis*, collected late in March, the spore mother cells had not yet assumed the spherical form. *S. cordata* and *S. glaucophylla*, collected at the same time, had already passed the tetrad stage.

The nucleus of the microspore divides some time before the spores are shed. The division of the nucleus is not followed by the formation of a cell wall. In *Populus monilifera*, representing the other genus of the Salicaceæ, a wall is formed separating a smaller lenticular cell from the larger one. In Salix the generative nucleus soon organizes a part of the surrounding cytoplasm and becomes a fusiform cell. Since spores already upon the stigmas showed no further differentia-



Pollen grains of *Populus monilifera*: *a* and *f*, before division of the nucleus; *b*, *c*, *d*, lenticular cell cut off from the rest of the spore; *e*, division of the nucleus of the larger cell. × 672.

tion, the division of the generative cell, which presumably takes place, although I was not so fortunate as to observe it, must occur after the pollen tube begins to form.

ORIGIN OF THE MACROSPORE.

The macrospore invariably has its origin in a hypodermal cell at or near the apex of the nucellus (*fig. 10*). Sometimes there are two or three hypodermal cells which by their size and intense staining indicate their sporogenous nature (*fig. 11*). A few cases were found in which two macrospores had developed to the fertilization stage, so it is evident that more than one of the sporogenous cells may continue its development. The usual appearance of an ovule before the differentiation of the archesporium is shown in *fig. 12*. In the nucellus six hypodermal cells, three of which are represented in the drawing, might be called archesporial cells, but I have not applied this term to a cell until it shows by its denser contents and reaction to stains that it has the characteristics of an archesporial cell.

The archesporial cell divides into a primary tapetal cell and a sporogenous cell which is the mother cell[2] of the macrospore (*fig. 13*). The primary tapetal cell sometimes gives rise to a tier of five or six cells, resulting in a deep placing of the macrospore. Usually there is a tier of two or three cells; but occasionally the primary tapetal cell does not divide (*figs. 14–19*). All of these variations were found in *Salix glaucophylla*, and an examination of several other species indicated a similar lack of uniformity.

The further development of the macrospore mother cell presents more important variations. Almost always it divides

[2] To avoid confusion this primary sporogenous cell will be called the *macrospore mother cell*. It is the cell in which the reduction of chromosomes takes place. If no tapetal cell is cut off, as in Lilium, Tulipa, and Fritillaria, the hypodermal archesporial cell becomes the macrospore mother cell without further division. If the macrospore mother cell divides into four, as in Polygonum, each of these is called a *potential macrospore*. If the macrospore mother cell gives rise to three cells or only two, each of these is a potential macrospore. In all cases the potential macrospore which matures is called a *fertile macrospore*.

into two cells, a smaller one nearer the micropyle, and the larger one which becomes the fertile macrospore. The smaller cell either undergoes one transverse division, thus giving rise to two potential macrospores, or it does not divide at all (*figs. 14* and *17*). In a case like *fig. 17* there is a possibility that the two smaller cells may have been cut off in succession from the larger cell, but as no mitotic figures were found in this stage this question could not be settled.

Sometimes the macrospore mother cell does not divide but develops directly into the macrospore (*fig. 23*). If any potential macrospores have been cut off, they are crowded and absorbed by the growing fertile macrospore until nothing remains of them but a refractive cap, and even this soon disappears. These variations are noteworthy. In Gamopetalæ it is said (21) to be the rule that the macrospore mother cell becomes the macrospore directly; in monocotyls and in the Archichlamydeæ among dicotyls the macrospore mother cell gives rise to four potential macrospores, one of which becomes the fertile macrospore. In some plants there are three potential macrospores; in others there are two; in still others the macrospore mother cell becomes the fertile macrospore without any division. Standard texts, as well as the original papers from which their information is obtained, leave the impression that for a given species there is little or no variation in the mode of origin of the macrospore, and I must confess that as far as the number of potential macrospores is concerned, I have noted the same uniformity in my study of Compositæ. But serial sections of about three hundred ovules of *S. glaucophylla* showed all of the above mentioned variations. *S. discolor* and other species indicated a similar variation. It is possible that generalizations have been based sometimes upon a few sections, and even these taken from the same plant. Such results are likely to be very uncertain, since individual plants often present variations from year to year. Not a single instance was found in which the fertile macrospore developed from the potential macrospore nearer the micropyle, as sometimes happens in Aster (28). The only sug-

gestion of such an occurrence is shown in *fig. 21*, and even here the usual cell has two nuclei. This preparation looks as if two potential macrospores might be developing one above the other.

GERMINATION OF THE MACROSPORE.

A typical nucellus just before the division of the primary nucleus of the fertile macrospore is represented in *fig. 14*. There is a tier of three tapetal cells and one potential macrospore, the latter already somewhat crowded by the growing fertile macrospore. The nucleus of the fertile macrospore is accompanied by the structures known as centrosomes. No attempt was made to investigate these bodies, but they were noticed in two other preparations. In *fig. 17*, which shows a portion of a nucellus, there is a tier of four tapetal cells and two potential macrospores. The first division of the primary nucleus of the fertile macrospore was observed in about forty cases. The most frequent appearance is that shown in *fig. 19*, which has a central strand of protoplasm traversing a vacuole and connecting the daughter nuclei. The vacuole may be absent, as in *fig. 18*. The spindle in the first division of the primary nucleus is parallel with the long axis of the macrospore, the only exception observed being that shown in *fig. 21*. In the second division several mitotic figures were found. The spindles at the micropylar end were always transverse to the long axis of the macrospore, while those of the antipodal end were always longitudinal. *Fig. 20* might fairly represent all the cases examined. In *fig. 16* the nuclei have a slightly different position, but it must be remembered that the nuclei in a germinating macrospore gradually change their position. A peculiar four-celled stage is shown in *fig. 22*, where the position of the nuclei and the size of the nucleus at the micropylar end would seem to indicate that after the first division the micropylar nucleus had failed to divide, while the nucleus at the antipodal end had divided and one of the resulting nuclei had divided again. The next division, giving four nuclei at each end of the sac, was observed in only two instances, neither of which was very satis-

factory. One of these (*fig. 23*) indicates that the longitudinal arrangement of spindles in the antipodal end and the transverse arrangement in the micropylar is continued in this stage. A portion of the contents, probably a micropylar spindle, has been washed out from this section, for the clear space between the mitotic figures is not a vacuole. It is common enough to find the eight-celled stage just as the two polar nuclei are fusing to form the primary endosperm nucleus. It would seem that after the second division the development proceeds rapidly, otherwise in examining a large number of sections from material representing stages from the first division of the primary nucleus to the eight-celled stage one should find the eight-celled stage as frequently as any other. As a matter of fact, the uninucleate condition is found most frequently; macrospores with two nuclei are not so frequent; those with four nuclei are comparatively rare; and those with eight nuclei are very exceptional. These observations show that the macrospore remains for some time in the uninucleate condition, a fact further indicated by the differences in the degree of maturity of ovules containing such macrospores. The increasing infrequency of the succeeding stages indicates that after germination has begun, development proceeds with increasing rapidity until the gametophyte has reached its fertilization period.

The macrospore may reach the eight-celled stage without increasing very much in size, or during the divisions which result in the four-celled and eight-celled stages there may be considerable enlargement at the expense of surrounding cells (compare *fig. 16* with *fig. 24*). After the eight-celled stage is reached, the macrospore increases greatly in size, as may be seen by comparing the figures of *pl. XIII* with those of *pl. XIV*, all of which are drawn to the same scale. This macrospore was a puzzle to me for more than a year. I had not yet found the third division resulting in the eight-celled stage, but the egg apparatus and primary endosperm nucleus seemed to demand such a stage. I could find no antipodals, and Treub's Casuarina (22) without antipodals added to the perplexity. An effort to con-

nect such stages as *fig. 16* and *fig. 28*, so as to account for a macrospore without antipodals, was unsatisfactory on account of the numerous instances in which nuclei were fusing to form the primary endosperm nucleus. A prolonged search revealed the missing antipodal cells (*figs. 26, 27*). A careful examination of about five hundred macrospores yielded six with indisputable antipodals and, since they are known to exist, other preparations show what may be reasonably interpreted as their disorganized remains. The antipodals are small, three in number, and are situated at the extreme chalazal end of the macrospore. In *fig. 31* the three cells marked *a* may be antipodals, for there does not appear to be any trace of a pollen tube or other evidence of fertilization, and the nucleus marked *e* looks like the primary endosperm nucleus rather than an endosperm nucleus resulting from its division. A somewhat similar condition is shown in *fig. 33*, but in this case there is a pollen tube already within the macrospore. If in either or both of these cases the cells marked *a* are cells of the endosperm, the primary endosperm nucleus has divided very early, and the appearance of the cells is unusual. It may be possible that the group of three cells has arisen from the division of the lower polar nucleus without any fusion with the upper one having taken place. If the nuclei belong to the endosperm, these two cases are the only ones observed in which there were more than two nuclei in the endosperm before the first division of the oospore.

In the the fusion of the polar nuclei to form the primary endosperm nucleus, details were not worked out, as Salix is not a favorable form. It may be noted merely that the fusion seems to be complete, even the nucleoli fusing to form one large nucleolus. A dense strand of protoplasm usually extends from the primary endosperm nucleus to the oosphere.

A few of the cells surrounding the chalazal end of the sac are very erythrophilous, probably through the influence of the antipodal cells.

It is very common to find the whole egg apparatus bursting through the apex of the nucellus into the micropyle. The

pointed ends of the synergids and sometimes the entire egg apparatus are thrust through the wall of the macrospore (*figs. 28, 30, 39*, and others).

The oosphere is sometimes spherical, but more frequently elongated and tapering slightly toward the micropylar end, which almost invariably contains a large vacuole. The nucleus is at the opposite end in a dense mass of protoplasm. In rare cases the oosphere is scarcely organized into a definite shape (*fig. 25*).

The synergids have their nuclei, which sometimes divide (*fig. 27*), and most of their protoplasm in the micropylar half, while the other half of the cell is almost entirely occupied by a large vacuole. The tips of the synergids frequently become covered by a strong wall which persists long after all other traces of the synergids have disappeared. These caps display considerable variation. There may be only a faint trace of striæ (*fig. 30*), or the striæ may be more prominent (*fig. 27*). Frequently the caps are so strongly developed that they give the synergids a decidedly beaked appearance (*figs. 28, 29, 36* and *39*), the beaks being cyanophilous, thus contrasting sharply with the prevailing erythrophilous structures of the macrospore.

Schacht (2) described such caps or "filiform apparatus" in *Santalum album*, but misinterpreted their relation to the synergids. Strasburger (18) some time afterward examined Santalum and mistook the cap for the entire synergid and the lower part of the synergid for an oosphere, thus getting a macrospore with two oospheres. He afterwards discovered his mistake and gave an excellent description of Santalum. Strasburger says that the caps contain minute pores through which there oozes an albuminoid substance which may attract the pollen tube. My preparations (*figs. 36* and *39*) support this view. In every instance in which beaks and pollen tubes were found, the pollen tube entered between the beaks. The beaks undoubtedly serve to enlarge the micropyle, thus facilitating the entrance of the tube. As a rule, the farther the egg apparatus is thrust beyond the nucellus the more strongly are the beaks developed. The function of

the beaks is probably to place the oosphere in a more favorable position and to attract and guide the pollen tube.

In the relative size of the nuclei and nucleoli of the primary endosperm nucleus, oosphere nucleus, and nuclei of the synergids the same uniformity was observed which characterized these structures in Aster. Over two hundred measurements in *Salix glaucophylla* gave the following results. The average length of the primary endosperm nucleus is 11.2 μ, and its breadth 10 μ; the diameter of the nucleolus being 5.7 μ. The oosphere nucleus is 8.8 μ long and 7.8 μ wide; and the diameter of its nucleolus 4 μ. The nuclei of the synergids are usually spherical, with an average diameter of 6.3 μ; and the diameter of their nucleoli 2.3 μ. All measurements were made from specimens which were ready for fertilization but had not yet been pollinated. The primary endosperm nucleus is always the largest, the oosphere nucleus next in size, and the synergid nuclei the smallest. The nucleoli have the same relative size. Measurements in other species gave the same relative results.

POLLEN TUBES AND FERTILIZATION.

In 1891 Treub (22) made the discovery that in Casuarina the pollen tube enters by way of the chalaza instead of the micropyle. In 1893 Betula was found to be chalazogamic, the discovery being made independently and almost simultaneously by Miss Benson (26) in England and Nawaschin (25) in Russia. Miss Benson at the same time added Alnus, Corylus, and Carpinus to the list, and Nawaschin soon added Juglans.

In consequence of these discoveries the pollen tubes of Salix were traced with considerable interest. In *S. glaucophylla, S. cordata, S. petiolaris,* and *S. tristis,* many pollen tubes were found, entering invariably by way of the micropyle. The chalazal region was examined critically in over three hundred ovules, but no trace of a pollen tube was found. The generative cell within the tube was observed but twice, and then under abnormal conditions (*fig. 38*). In a few cases the male cell was observed within the oosphere. The nearest approach to conjugation which

my preparations afforded is shown in *fig. 40*. As the pollen tube enters the sac the synergids usually break down, and even their nuclei disappear. A sac immediately after fertilization is shown in *fig. 32*, in which the oospore is much enlarged and is forming a cellulose wall, and only a nearly disintegrated nucleus and a mass of protoplasm mark the remains of the synergids. The primary endosperm nucleus has not yet divided. A typical case is shown in *fig. 36*, which represents the pollen tube entering between the beaks of the synergids. The fusion of sex cells has probably not taken place, for no membrane has yet formed around the oosphere. The primary endosperm nucleus has increased greatly in size, but has not divided. The enlargement of the endosperm nucleus before fertilization is also shown in *fig. 34*. A somewhat later stage is given in *fig. 39*. The pollen tube can be seen still between the beaks of the synergids. The oospore has its cellulose wall, but the primary endosperm nucleus, although greatly enlarged as usual, has not yet divided, this enlargement beginning before the pollen tube reaches the beaks of the synergids.

A very peculiar case is represented in *fig. 38*, in which the embryo is quite advanced, but both synergids still persist. These synergids are plump and have definite cell walls, but have no vacuoles, and their nuclei are at the lower end instead of the upper where they are usually situated. The pollen tube, of course, is not the one which assisted in fertilization. A similar condition is shown in *fig. 35*, but here the synergids have no walls, and the pollen tube has collapsed. These cases indicate that fertilization may take place without the assistance of the synergids. Another singular case is furnished by *fig. 37*, in which the embryo is quite advanced, but the primary endosperm nucleus, although it has grown very large, has not yet divided.

As a rule, the division of the primary endosperm nucleus precedes the division of the oospore, and for a short time the nuclei of the endosperm multiply more rapidly than the cells of the embryo. A two-celled embryo is usually correlated with four nuclei in the endosperm, a four-celled embryo with eight or

ten cells in the endosperm, but the endosperm does not continue to keep pace, and very soon the cells of the embryo outnumber the nuclei of the endosperm. The nuclei of the endosperm in Salix are never separated by cell walls.

DEVELOPMENT OF THE EMBRYO.

The first division of the oospore is always transverse to the longer axis of the embryo sac (*figs. 41, 42*). Occasionally this division separates the oospore into approximately equal parts, but it is more usual to find the suspensor cell larger and somewhat tapering, while its sister cell, which gives rise to the greater part of the embryo, is uniformly hemispherical. The suspensor cell divides transversely, and the daughter nuclei pass into the resting stage with full sized nucleoli before the embryo cell divides (*fig. 43*). The first division of the embryo cell is always longitudinal (*figs. 44–46*). The literature of the subject indicates that this division is almost universal in angiosperms, if we except those which have no suspensor and those in which the suspensor, though present, contributes nothing to the embryo. For instance, in Capsella after the first division of the oospore, the cell nearer the micropyle undergoes several divisions, forming the long suspensor, while its sister cell remains passive until the first longitudinal division occurs. This seems to be mere assumption, but it is quite probable that in dicotyls the terminal cell in which the first longitudinal division appears gives rise to the greater part of the embryo. Vines in his *Text-Book* has unfortunately figured the first division of the embryo cell in the type Capsella as transverse. The figures are diagrammatic "after Goebel and Hanstein" but Hanstein (3) figures the first division as vertical, and Goebel has followed him. Vines' text, however, without any particular reference to Capsella, states that the first division is usually longitudinal. Some of Hanstein's figures, like his *figs. 9* and *11*, which show a complete differentiation of the dermatogen before the first vertical division, certainly need to be verified, especially since the drawings were made from embryos squeezed out from the ovule and rendered transparent, a process which

might cause one to lose a cell wall now and then. I have examined over twenty cases of the first division of the embryo in Capsella, and classes in the laboratory have thoroughly examined this and other early divisions in the same type, but have found no exception to the rule that the first division is longitudinal. Hanstein does figure the first division as transverse in Nicotiana and *Viola altaica*, but the figures are not convincing because the three nuclei in the large upper cell of his *figs. 7* and *9* of *pl. 5* make it possible to apply the usual interpretation.

In Salix, as a rule, the second division is also longitudinal and at right angles to the first, but it occasionally happens that the second division is transverse (*figs. 48, 51*). Both cases may be found in the same species, and in *S. cordata* and *S. petiolaris* I have found both on a single plant. In studying sections of embryos in these early stages, it is very easy to make mistakes. The young walls are often elusive, even in good preparations, and it is safest to make the sections thick enough to include the whole embryo. The nuclei will then enable one to interpret with certainty such stages as *figs. 48, 49, 53*.

The third division, usually transverse, but sometimes longitudinal, brings the embryo into the familiar octant stage (*figs 53, 54*). The first transverse division separates the hypocotyl and cotyledon portions of the embryo.

After the octant stage one naturally looks for the periclines which mark off the dermatogen, and usually they are found, but the embryo sometimes proceeds a little further before this differentiation takes place. Sometimes a pericline cuts off the dermatogen in one octant, while a neighboring octant makes one or more divisions before the pericline appears (*figs. 56, 59*). In Capsella the first pericline usually appears in the upper octants; in Salix I can find no regularity, the first pericline appearing in one octant as frequently as in another. The entire dermatogen, exclusive of the suspensor contribution, may be cut off while the whole embryo consists of only sixteen cells. Very rarely, a part of the dermatogen is cut off while the embryo is still in the quadrant stage. An interesting stage is shown in *fig. 65*, which

has, beside the dermatogen, sixteen cells which are to become differentiated into periblem and plerome. Some writers say that the periblem and plerome are differentiated very early, and they have even pointed out the first cell which is to produce plerome and the first which is to produce periblem, as if each cell were predestined to play a certain rôle. Hanstein's (3) classic account of Capsella, followed by the standard textbooks, illustrates this idea; Fleischer (7) is equally definite in his description of Ornithogalum and Viola; and there is no doubt that their figures are accurate. Everyone who has cut Capsella knows how easy it is to duplicate most of Hanstein's figures. It is possible, perhaps probable, that the theory is correct in the case of Capsella, as it has a very regular embryo. In the other types which Hanstein considers, such an explanation is not so satisfactory. Fortunately, he does not attempt to apply the theory to all plants. Fleischer would apply it to dicotyls in general, but in his Asclepias one cannot distinguish periblem from plerome in early stages. It is evident that monocotyls, in many of which the plerome can hardly be called an independent system, must have a different explanation.

In the more regular embryos of Salix a person with some ingenuity might imagine this early differentiation into periblem and plerome, but the usual forms would demand some other theory. In Salix there are no four cells, which with their posterity are predestined to form the plerome of the plant, as in Hanstein's Capsella, but, as will be shown, the differentiation of these tissues occurs very late in the development of the embryo.

The relation between the suspensor and embryo in early stages is shown in *figs. 68, 70, 73*. It will be seen that the upper cell of the suspensor has divided by a longitudinal wall. A second longitudinal division, which may take place in embryos even younger than these, divides the upper cell of the suspensor into a plate of four cells (*fig. 53*). The dermatogen of the whole embryo, except the part contributed by the suspensor, is differentiated in embryos still younger than that shown in *fig. 65*. The dermatogen is the first of the primary tissues of the root tip

to be differentiated, the first step in this differentiation being marked by the spindle in *fig. 77*. This division completes the dermatogen of the root tip, joining it with the dermatogen of the rest of the embryo, and furnishing the first layer of the root cap (*figs. 72, 75, 77a*). These figures show no differentiation into periblem and plerome. I do not believe that the suspensor contributes anything to the periblem in Salix. An embryo almost in the cotyledon stage (*fig. 74*) shows a complete dermatogen, but still no definite plerome and periblem. Nearly mature embryos (*fig. 66*) have the periblem and plerome sharply differentiated a short distance above the dermatogen of the root cap, but are indistinguishable at the apex, and both tissues still come from a common meristem. This figure represents the characters of the various regions of late embryos. The plerome cells are marked by dense protoplasmic contents free from vacuoles. Except very near the meristem they are elongated, and their long nuclei usually have two or more nucleoli. It is a region of cell elongation rather than of cell division. The periblem cells with their numerous vacuoles, spherical nuclei, and looser arrangement, present a noticeable contrast, which is emphasized by the fact that they are broader than long, and show evidences of cell multiplication rather than elongation. The prevailing divisions are transverse. The cells of the hypodermal layer of the periblem soon become sharply differentiated. The protoplasm with its nucleus is crowded against the inner wall of the cell by the encroaching vacuoles, which merge into one large vacuole containing a substance which seems to be suberin. A transverse section of the plerome and part of the periblem at this stage is represented in *fig. 69*. In *fig. 76* the periblem and plerome seem to be completely differentiated. At the apex there is only one layer of periblem between the plerome and dermatogen and this is usually the case in mature embryos. This figure also shows the usual appearance of the layers of the root cap. The root region of an embryo which has completed its intraseminal development has a separate meristem for the periblem and plerome (*fig. 67*, the plerome and dermatogen being shaded, and

the initial cell of the plerome with one of its segments being more deeply shaded).

Thus it is seen that in very young embryos all the cells are meristematic, and no tissues are differentiated. The first tissue to differentiate is the dermatogen, the greater part of which is usually cut off immediately after the octant stage. Some time before the appearance of the cotyledons the dermatogen is completed by a contribution from the suspensor. The periblem and plerome, which are indistinguishable at the apex and grow from a common meristem during the greater part of their intraseminal development, become completely differentiated and grow from separate initials before the intraseminal development is completed.

It must be remembered that the development of the primary root of an embryo, in which the suspensor usually plays such an important part, is a very different thing from the development of a lateral root which is not modified by any suspensor contribution.

The suspensor presents some variation, as may be seen by comparing the figures of *pl. XVI.* After the suspensor has reached the three or four-celled condition, which it does at a very early stage, its cells stop dividing until the dermatogen is cut off to complete the dermatogen of the root. The middle cells of the suspensor, *i. e.*, the one or two cells below the hypophysis, then divide and sometimes give rise to eight or ten cells. The suspensor cell nearer the micropyle does not seem to divide.

A glance at such embryos as those represented in *figs. 71* and *73* will show that the development below the first transverse division of the embryo is more regular and symmetrical than that of the upper half. In the hypocotyledonary portion there is a zone of cells (*z, figs. 71, 73*) which is frequently quite conspicuous at this stage. Below this zone the same figures show that the arrangement may be somewhat symmetrical. Even in the upper part, an embryo as regular as that drawn in *fig. 71* shows some symmetry in the arrangement of its cells, but usually there is no regularity or symmetry except in the general outline. I have

made no special study of the upper part of embryos older than that represented by this figure. The embryo loses its spherical or ovoid form, becomes flat across the top, two regions of more rapid cell division and growth appear which push the cotyledons up above the less active meristem of the main axis, and the embryo assumes the characteristic form shown in *fig. 66a.*

No account of Salix would be complete without mentioning peculiar embryos which depart from the normal course of development and for a time seem to have an apical cell. In one of these embryos (*fig. 61*) the apical cell is three-sided, and has cut off two segments in true pteridophyte fashion. A surface view of another is shown in *fig. 64*, and a median section of the same embryo is given in *fig. 63*, while still another peculiar embryo is shown in *fig. 62*. No trace of such apical cells is found in embryos older than these. If such embryos mature, it would be interesting to discover how the periblem and plerome differentiate, and what part the suspensor plays in the development.

TERATOLOGY.

Salix has been notable always for the frequency and variety of its sports. It is now monosporangiate and diœcious, but embryology gives no evidence that this is due to suppression, suggesting rather that it represents a primitive condition.

A vigorous plant of *S. glaucophylla* was found in the spring of 1895, many of the pistillate catkins of which were three or four inches long. A few catkins were entirely staminate, others were entirely pistillate, but many were mixed, some of the bracts having two stamens, some having one pistil, others having one pistil and one stamen, and still others having one pistil and two stamens. The pollen and stigmas matured at about the same time. Sections of the pistils showed perfectly normal conditions from the origin of the macrospore to the mature seed. The plant behaved the same way the next spring, and buds collected during the past winter showed that the same peculiarities will be continued. I have planted seeds to discover whether these characters can be propagated in that way.

A plant of *S. cordata* had some bracts with two pistils, and some with one pedicel bearing two pistils at its tip, but nearly all the bracts had the usual single pistil. No stamens were found upon this plant. Sections showed normal ovules and embryo development.

A plant of *S. petiolaris* found in the spring of 1896 exhibits the most surprising variety of sports. On this plant were found both staminate and pistillate catkins, catkins with pistils from some bracts and stamens from others, also catkins in which two stamens and one pistil, or one stamen and one pistil came from the axil of the same bract. Sections of material from this plant revealed interesting monstrosities, which are almost endless in their variety. For the sake of comparison, a section of a normal pistil of the same species, drawn to the same scale, is given in *fig. 78*. Sections like *fig. 83* were not uncommon. Externally this pistil seems perfectly normal, but at the base of the ovary there is a single ovule instead of the half-dozen or more which are expected in this species. The embryo sac shows a well developed egg apparatus and primary endosperm nucleus. A single erect microsporangium is borne upon a stalk which closely resembles the placenta which bears the ovules. In *fig. 88* there is external irregularity in the position of the stigma. The ovules are normal, one having a perfect embryo with the usual amount of endosperm, and the other having a well developed embryo sac. The single microsporangium is not borne upon a stalk, but nearly upon the wall of the carpel. In *fig. 79* there are four ovules at the base of the ovary, all with embryo sacs developed to the fertilization stage. At the upper part is an ovule placed transversely. The middle is occupied by four microsporangia of very different aspect, one being borne upon a long slender stalk, another just above it having a somewhat placental base and decidedly pointed apex, while one of those on the other side is borne on the wall of the carpel, and the other upon a pla cental growth developed at a fold in the carpel. In *fig. 82* there are two pistils upon a single pedicel, in one of which there is but a single poorly developed ovule, in the other two normal ovules

and two microsporangia. In *fig. 85* the two pistils are united for half their length, one having two feebly developed microsporangia and one normal ovule, and the other the lower ovule perfectly orthotropous and with a perfect integument all around, its embryo sac being normal. This ovule is borne upon a long, smooth, slender stalk, which springs from the usual placental outgrowth. These long stalks were observed several times, and they always bore orthotropous ovules. It will be remembered that the anatropous or orthotropous character of ovules is used as a taxonomic character, the normal ovules of *Salix* being anatropous. The other ovule is anatropous, and presents nothing exceptional except that the placental outgrowth is elongated. Another orthotropous ovule is shown in *fig. 87*, one of the two microsporangia having a long stalk. In *fig. 86* one might fairly claim an ambisporangiate flower. The pistil contains two normal ovules, and one ovule curiously formed in the wall of the carpel, while the upper part of the ovary is occupied by two large microsporangia, one of which is not represented. The staminate flower, if such it may be called, has two microsporangia lying side by side, one of which is not represented. The stalk has the structure of a carpel wall rather than that of a filament. In *figs. 80, 81* we have utterly irregular conditions. The ovules are not at all enclosed in the ovary, three of them being borne transversely and one of them orthotropous. Two of the embryo sacs were normally developed and look as if they might produce embryos. This would afford an instance of fertilization in angiosperms without the intervention of a stigma. The pollen could fall directly upon the ovule and a very short pollen tube would suffice. Such open carpels are not rare in this plant and it is probable that a careful search would yield cases of fertilization and embryo formation. A curious case is shown in *fig. 84*, where a common stalk branches into two filaments, each bearing an anther. Each anther has four microsporangia, two longer and larger on the inner side, and two spherical ones on the other side of the connective. In the anther on the right, the connective is prolonged into a well developed stigma.

Examples might be multiplied almost indefinitely, but these illustrate the general direction of the irregularities. Monosporangiate and ambisporangiate flowers in Salix have been described before, but I can find no account of microsporangia borne inside the ovary, or of orthotropous ovules.

The more minute anatomy deserves some attention. As a rule, the macrospores have a perfectly normal development. Most of the material showed the macrosporangia at the fertilization period, and the egg apparatus and primary endosperm nucleus could not be distinguished from those of normal plants, and in several cases, as in *fig. 88*, embryos were developing in the usual way. The stamens of monosporangiate flowers, as well as those of the ambisporangiate flowers, developed exactly like other stamens in every detail which I was able to observe, but the microsporangia which were borne within the ovary need separate mention. These sporangia were usually solitary, but sometimes in pairs, and the wall usually had no layer at all between the tapetum and endothecium, the former often being abnormally developed, as in *fig. 3*. It is not at all unusual to find cells of the tapetum with two, three, or even four large nuclei, as represented in this figure. This preparation also shows cells of the tapetum which have divided by periclines. The cells of the sporogenous tissue are irregular in shape and probably would not have developed spores. Another irregular case is shown in *fig. 6*, where the sporogenous cells, probably spore mother cells, have surrounded themselves with a thick wall. Instances like *figs. 3* and *6* are common, where the sporangium development is feeble and seems to have been checked. Many of the microsporangia, however, especially those which are more or less stalked, present a more normal development. A characteristic example of the microsporangia which continue their development is seen in *fig. 7*, the wall appearing much like that in *fig. 8*, which is drawn from a perfectly normal anther of *S. cordata*. The pollen grains are somewhat vacuolated (as are the cells of the tapetum), and show the division into tube nucleus and generative nucleus, which are slightly smaller than is usually the case in *S. petiolaris*,

but the pollen grains in *fig. 5* could not be distinguished from normal ones at this stage. The pollen grains continue their development, the generative nucleus organizing a part of the surrounding cytoplasm and becoming the center of a fusiform cell (*fig. 4*). It is hardly probable that the pollen of these internal microsporangia plays any part in fertilization, for it is uniformly later in developing than the macrospores.

Those who regard Salix as a reduced type rather than a primitive one might consider this mixture of monosporangiate and ambisporangiate forms as favorable testimony, but they furnish better evidence that even such variations as a change from diœcism to monœcism or even to an ambisporangiate condition may appear suddenly. The orthotropous ovules and microsporangia inside of the ovary are also suggestive.

SALIX AND OTHER AMENTIFERÆ.

The occasional presence of more than one macrospore in Salix is in harmony with what is known of other Amentiferæ. A few preparations of early stages in *Populus tremuloides* show five or six cells which are elongated to three or four times the length of the surrounding ones, have richer contents, and appear to have equally good prospects of becoming macrospores.

The early development of the macrospore agrees more nearly with Nawaschin's Betula than with any other of the described Amentiferæ.

The tracheids, which form such a marked feature in Treub's Casuarina and Miss Benson's Castanea, do not occur in Salix.

Salix has no cæcum, unless the elongated antipodal end of the sac can be regarded as such. Cæca are so prevalent in Casuarina and the British Amentiferæ that Miss Benson says "they may fairly be regarded as of taxonomic value."

The embryo sac of Salix, at the fertilization period, differs from those of Alnus, Corylus, Betula, Carpinus, Juglans, and Myrica, in that these have antipodals which may be found with some ease, in some of them the antipodals being quite persistent and forming thick cellulose walls. I am inclined to think that

Treub's Casuarina agrees with Salix in that its antipodals are also hard to find. Treub states that they do not exist, and in claiming the development of an embryo sac without antipodals he has certainly given us something unique. Treub's main work was upon the sporogenous tissue, sterile macrospores, and chalazogamy, and his results here are unquestionable; but it might be worth while to have the development of the macrospore worked out in detail.

With the exception of the problematical case represented in *fig. 31*, nothing was observed which would suggest the formation of endosperm before the entrance of the pollen tube. In Casuarina, as described by Treub (22), the endosperm is formed before fertilization, and does not have its origin in a primary endosperm nucleus formed by fusion of polar nuclei. If Casuarina has no primary endosperm nucleus, the mode of origin of the endosperm is also unique. The formation of endosperm before fertilization is not at all unusual, if fertilization be defined strictly as the fusion of the sex cells. In general the division of the primary endosperm nucleus precedes the division of the oospore as frequently as it follows, and it is not exceptional to find two or four nuclei in the endosperm before the division of the oospore, but in all these cases the formation of endosperm seems to be initiated through the influence of the pollen tube. Since Treub's figures show the pollen tube within the macrospore he may have merely an unusual amount of endosperm formed before the fusion of the sex cells. It is certainly true that Casuarina has a more extensive endosperm formed before the division of the oospore than has yet been described for any other angiosperm, Myrica somewhat approaching it in this respect. Unfortunately I have had no opportunity to examine any preparation of Casuarina.

RECAPITULATION AND SUMMARY.

Complete series were studied in *Salix glaucophylla*, *S. petiolaris*, *S. cordata*, and *S. tristis*, with less complete series in thirteen other species.

1. *Organogeny of the flower.*— Pistillate buds, collected in August show the carpels outlined but no trace of ovules. October buds of *S. glaucophylla* and *S. cordata* show the nucellus, but the integument as a rule does not appear until spring. Staminate buds collected in October show the stamens well outlined. The nectaries in both staminate and pistillate buds can be seen in October. A diligent search failed to reveal the slightest trace of rudimentary floral organs, which those who regard Salix as a reduced type might expect to find.

2. *Development of the microspores.*— A comparison of autumn, winter, and early spring buds shows that most stamens pass the winter in the spore mother cell stage. The division into generative nucleus and tube nucleus takes place before the tapetum breaks down. The generative nucleus soon organizes a part of the surrounding cytoplasm and becomes a fusiform cell. No wall is formed between the nuclei. *Populus monilifera* differs in this respect, a definite wall separating the two cells. The cells of the tapetum are often binucleate.

3. *Origin of the macrospore.*—The macrospore has its origin in a hypodermal cell at the apex of the nucellus. Sometimes there are two or three archesporial cells, but it is very seldom that more than one develops. The primary tapetal cell usually gives rise to a tier of three or four cells, but sometimes does not divide. The macrospore mother cell usually cuts off one or two potential macrospores, but sometimes germinates without cutting off any such cells. This variation is prevalent in the genus.

4. *Germination of the macrospore.*— The first division of the primary nucleus of the macrospore is transverse. In the second and third divisions the spindles at the micropylar end are transverse, while the spindles at the antipodal end are longitudinal. After the first division, development proceeds with increasing rapidity until the female gametophyte has reached the fertilization period. Great difficulty was experienced in demonstrating the presence of antipodal cells, several hundred macrospores, just before the fertilization period, yielding only six cases of

undoubted antipodals. This might suggest that Casuarina may have antipodals which are also evanescent and hard to find.

The synergids frequently have a strongly developed "filiform apparatus," which gives them a beaked appearance. The egg apparatus breaks through the wall of the macrospore and projects into the micropyle. In a few cases the synergids were observed to persist until the embryo was almost in the cotyledon stage.

5. *The pollen tubes and fertilization.*—The pollen tubes were examined with great care in several species on account of the discovery of chalazogamy in several of the Amentiferæ, but in every case the pollen tube was observed to enter the micropyle. The beaks of the synergids open the micropyle and attract the pollen tube.

The generative nucleus was observed in the pollen tube and in the oosphere, but not in the act of fusion. The polar nuclei fuse to form the primary endosperm nucleus before the fusion of sex cells takes place. As soon as the pollen tube enters the micropyle the primary endosperm nucleus begins to enlarge, and its division usually precedes that of the oospore. In one case the embryo had almost reached the cotyledon stage and the primary endosperm nucleus had not yet divided.

6. *Development of the embryo.*—The first division of the oospore is always transverse and that of the embryo cell is always longitudinal. The second division is usually longitudinal, but sometimes transverse, and the third division usually transverse but sometimes longitudinal. The differentiation of dermatogen usually immediately follows the octant state. The first pericline cutting off dermatogen appears in one quadrant as frequently as in another. Sometimes an octant will make one or two other divisions before the dermatogen is cut off. The dermatogen of the root tip is contributed by the upper cell of the suspensor. The suspensor does not contribute anything to the periblem. Periblem and plerome cannot be distinguished in early stages, as in Capsella. For a time, periblem and plerome grow from a common meristem, but toward the close of intra-

seminal development they become differentiated even at the apex and grow from separate initials.

7. *Teratology.*— In addition to monosporangiate and ambisporangiate forms, which have been described by other observers, a strange sport of *S. petiolaris* was found with microsporangia growing within the ovary. Sometimes the microsporangia were upon long stalks, sometimes upon placentalike outgrowths of the carpel, and sometimes imbedded in the carpel wall. One case showed two quadrilocular stamens with the filaments united below, and the connective prolonged above into a stigma. In the microsporangia borne inside the ovaries the microspore development was sometimes normal, but was as often feeble and abortive. In ovaries which contained microsporangia the ovules were sometimes perfectly orthotropous, and had the integument developed all around. The macrospore development was normal and embryos were not uncommon. Collections, representing in some cases three flowering seasons, show that a plant may continue its particular sport year after year.

8. *Salix and other Amentiferæ.*—Salix does not have the extensive archesporial tissue in the ovule described for several Amentiferæ, but sometimes has two or three archesporial cells. The development of the macrospore agrees more nearly with Betula than with any other of the described Amentiferæ. There are no nucellar tracheids as in Castanea and Casuarina. The difficulty in finding antipodals in Salix would suggest that the development of the macrospore in Casuarina be reinvestigated.

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EXPLANATION OF PLATES XII–XVIII.

List of abbreviations used: *a*, antipodals; *b*, beak, or filiform apparatus; *c*, root cap; *d*, dermatogen: *em*, embryo; *en*, primary endosperm nucleus; *m*, macrospore mother cell; *mg*, male generative cell; *o*, oosphere; *on*, oosphere nucleus; *per*, periblem; *pl*, plerome; *pt*, pollen tube; *syn*, synergid; *t*, tapetal cell.

All figures, except those of *pl. XVIII* and *fig. 66a*, were drawn with a $\frac{1}{12}$ Bausch and Lomb immersion and Zeiss ocular no. 4.

Figs. 1–9, $\times$ 594; figs. 10–24, $\times$ 631; figs. 25–65, $\times$ 694; figs. 66–77*a*, $\times$ 390; figs. 78–88, $\times$ 40.

PLATE XII.

FIG. 1. Young anther of *Salix glaucophylla*. October 1.

FIG. 2. Young anther of *Salix tristis*. March 31.

FIG. 3. *S. petiolaris*. Young microsporangium of a sport.

FIG. 4. *S. petiolaris.* Mature pollen grains of a sport.

FIG. 5. *S. petiolaris.* Portion of microsporangium of sport.

FIG. 6. *S. petiolaris.* Portion of microsporangium of sport. Irregular development.

FIG. 7. *S. petiolaris.* Portion of microsporangium of sport.

FIG. 8. *S. cordata.* Portion of microsporangium of normal anther.

FIG. 9. *S. cordata.* Later stage of development than *fig. 8.*

PLATE XIII.

S. glaucophylla.

FIG. 10. Apex of nucellus with single archesporial cell.

FIG. 11. Apex of nucellus with two archesporial cells.

FIG. 12. Apex of nucellus before the differentiation of the archesporium.

FIG. 13. Nucellus showing macrospore mother cell and primary tapetal cell.

FIG. 14. Typical nucellus with one fertile macrospore, one potential macrospore and three tapetal cells. The nucleus of the fertile macrospore is accompanied by two centrosomes.

FIG. 15. First division of the primary nucleus of the macrospore; one potential macrospore; two tapetal cells.

FIG. 16. Second division; one potential macrospore; one tapetal cell.

FIG. 17. One fertile macrospore; two potential macrospores; four tapetal cells.

FIGS. 18, 19. First division; one potential macrospore; three tapetal cells.

FIG. 20. Second division, showing transverse micropylar spindle and longitudinal antipodal spindle.

FIG. 21. Irregular development of macrospore.

FIG. 22. Irregular development; the micropylar nucleus has probably not divided.

FIG. 23. Third division showing position of spindles. One spindle or pair of nuclei has washed out.

FIG. 24. Second division; unusual destruction of nucellar tissue for this stage.

PLATE XIV.

FIG. 25. *S. glaucophylla.* Oosphere indefinite in form; nucleolus of primary endosperm nucleus very dense; synergid nuclei distinct; filiform apparatus well developed.

FIG. 26. *S. glaucophylla.* Antipodal region; three definite antipodals.

FIG. 27. *S. glaucophylla.* Three antipodals; fusion of polar nuclei to form primary endosperm nucleus; one synergid with two nuclei.

FIG. 28. *S. petiolaris.* Egg apparatus projecting from nucellus; the synergids sharply beaked.

FIG. 29. *S. glaucophylla.* Extreme development of the beak or "filiform apparatus."

FIG. 30. *S. petiolaris.* Egg apparatus projecting; filiform apparatus feebly developed.

FIG. 31. *S. glaucophylla.* Cells marked *a* may be three antipodals; primary endosperm nucleus apparently not divided; if not antipodals the three cells may have resulted from the lower polar nucleus and the nucleus (*en*) may not be the result of fusion.

PLATE XV.

FIG. 32. *S. glaucophylla.* Just after fertilization; oospore is enlarged and has cellulose wall; endosperm nucleus not yet divided.

FIG. 33. *S. petiolaris.* Three cells marked *a* may be antipodals, or may explained as in *fig. 31.*

FIG. 34. *S. petiolaris.* Pollen tube has entered but fusion has not yet taken place; endosperm nucleus has become very large.

FIG. 35. *S. petiolaris.* Synergid persisting long after fertilization.

FIG. 36. *S. petiolaris.* Pollen tube entering between beaks of synergids; fusion not yet effected; primary endosperm nucleus very large.

FIG. 37. *S. glaucophylla.* Primary endosperm nucleus not yet divided, an abnormal delay.

FIG. 38. *S. glaucophylla.* Unusual persistence of synergids; synergids have no vacuoles and their nuclei are in an unusual position; the pollen tube, of course, is not the one which was concerned in fertilization; endosperm forming in the usual manner.

FIG. 39. *S. petiolaris.* Pollen tube between beaks of synergids; fusion has taken place, oospore very spherical; primary endosperm nucleus not yet divided.

FIG. 40. *S. glaucophylla.* Entrance of male generative nucleus; this nucleus is lenticular and its volume is less than that of the oosphere nucleus, although the figure gives a contrary impression.

PLATE XVI.

FIG. 41. *S. petiolaris.* First division of oospore.

FIG. 42. *S. glaucophylla.* First division of oospore.

FIG. 43. *S. petiolaris.* The suspensor cell has divided.

FIGS. 44–46. *S. petiolaris.* First division of the embryo.

FIG. 47. *S. petiolaris.* Embryo of three cells; upper cell of suspensor has divided.

FIG. 48. *S. petiolaris.* Quadrant stage; second division of the embryo has been transverse; all nuclei are shown; synergid persisting.

FIG. 49. *S. cordata.* Quadrant stage; second division of embryo longitudinal.

FIG. 50. *S. petiolaris.* Quadrant stage; second division of embryo longitudinal.

FIGS. 51, 52. Quadrant stages in *S. petiolaris* and *S. cordata* respectively.

FIG. 53. *S. petiolaris.* Octant stage; upper cell of suspensor has given rise to a plate of four cells; synergid persisting.

FIG. 54. *S. petiolaris.* Quadrant stage: embryo small as compared with the suspensor.

FIG. 55. *S. petiolaris.* Irregular embryo.

FIG. 56. *S. cordata.* Dermatogen cut off in one segment while a neighboring segment is developing farther before cutting off dermatogen.

FIG. 57. *S. petiolaris.* Lower cell of suspensor much enlarged.

FIG. 58. *S. cordata.* Irregular embryo.

FIG. 59. *S. petiolaris.* Embryo of sport; variation in the stage at which dermatogen is cut off.

FIG. 60. *S. cordata.* Early division in middle cell of suspensor.

FIG. 61. *S. petiolaris.* Three-sided apical cell.

FIG. 62. *S. cordata.* Apical cell.

FIG. 63. *S. cordata.* Later stage of embryo which started to develop by an apical cell.

FIG. 64. Surface view of same embryo as *fig. 63*.

FIG. 65. *S. glaucophylla.* All dermatogen cut off except the suspensor contribution; sixteen cells in the embryo besides the dermatogen.

PLATE XVII.

FIG. 66. *S. tristis.* Root end of nearly mature embryo, showing character of various cells; periblem and plerome not entirely differentiated at the apex.

FIG. 66*a*. Outline sketch of same embryo showing cotyledons and apex of stem.

FIG. 67. *S. tristis.* Periblem and plerome differentiated even at the apex; initial cell of plerome and one segment more deeply shaded; dermatogen and four layers of root cap also shown.

FIG. 68. *S. glaucophylla.* Embryo before differentiation of the dermatogen of the root tip.

FIG. 69. *S. tristis.* Transverse section of embryo in the stage shown in *fig. 66,* taken a few cells above the common meristem of periblem and plerome.

FIG. 70. *S. glaucophylla.* Embryo unusually symmetrical in its divisions.

FIG. 71. *S. glaucophylla.* Very symmetrical embryo showing the zone of cells (*z*) just below the first transverse wall.

FIG. 72. *S. tristis.* Dermatogen of root tip differentiated; no trace of separation into periblem and plerome.

FIG. 73. *S. glaucophylla.* Embryo showing zone of cells (*z*); development more symmetrical in hypocotyledonary portion than in the cotyledonary.

CHAMBERLAIN on SALIX.